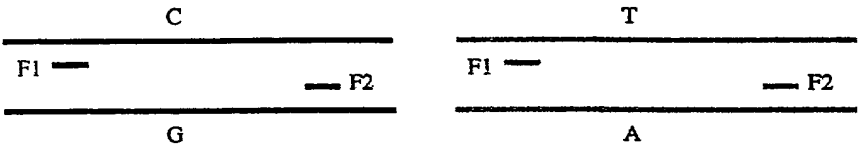
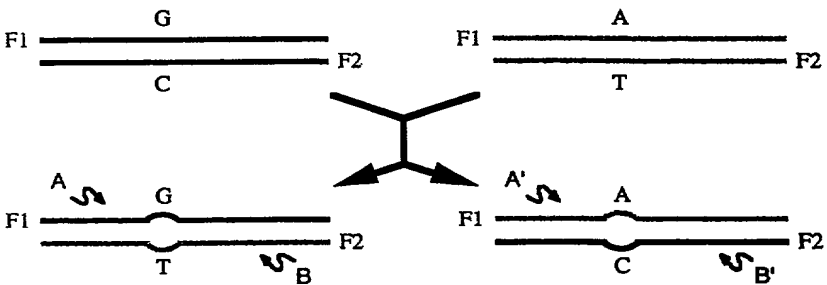


Mismatch scanning Assay. (Endo V / DNA Ligase)

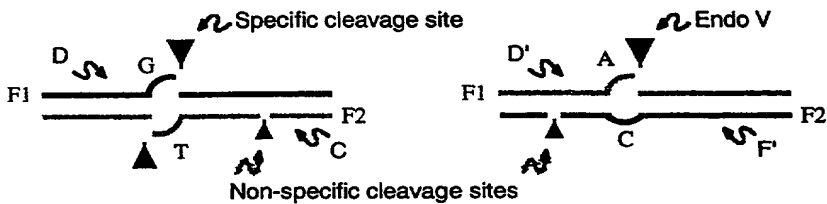
1. PCR amplify gene using primers with different fluorescent labels and *Taq* DNA polymerase.



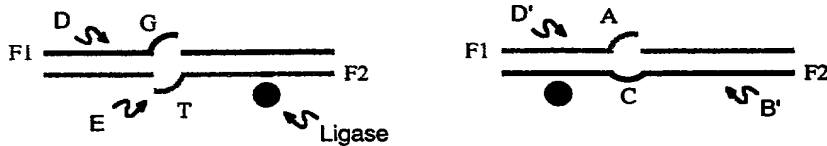
2. Denature and reanneal PCR products to form heteroduplexed DNA. (Homoduplexed products not shown).



3. Preferentially nick DNA one base to the 3' side of mismatches using thermostable Endonuclease V.



4. Add thermostable ligase to re-seal background nicks at perfect match regions.



	1.	2.	3.
EndoV	-	+	+
Ligase	-	-	+

5. Separate fluorescent products on a DNA sequencer (using length standards) to approximate site of mismatch.

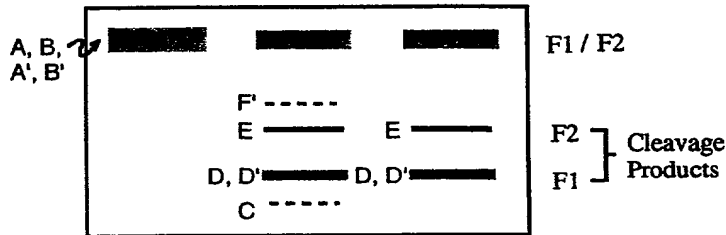
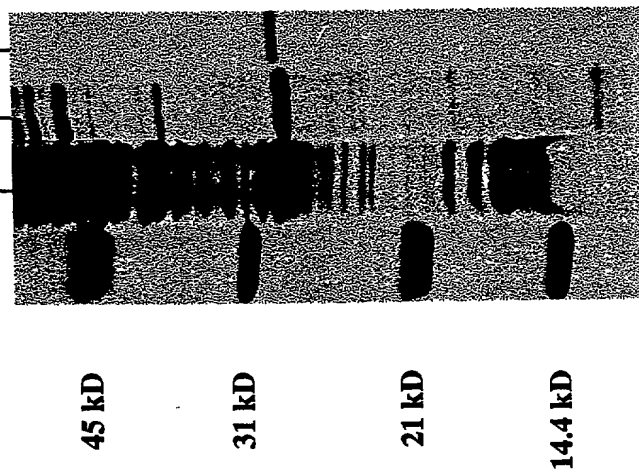


Figure 1

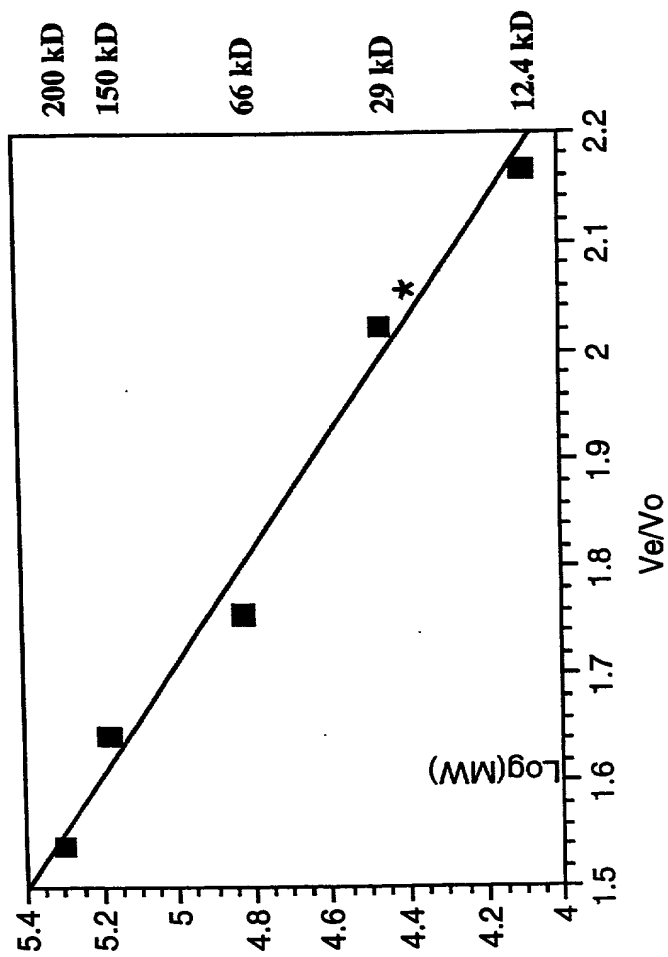
75°C 15'

Crude extract

SP Column



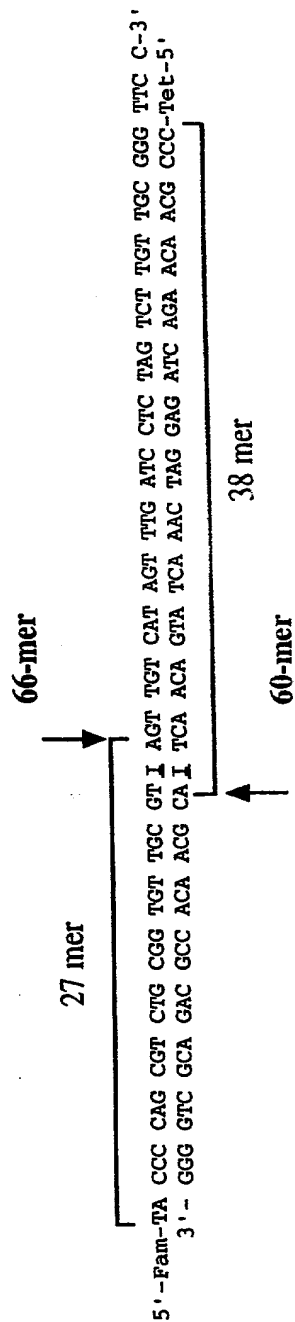
A



B

Figure 2

A



E:S = 1:10, S = 10 nM Mg^{2+}

F/H
C/H
G/H
A/H
I/F
I/C
I/G
I/A
I/I
I/I



E:S = 10:1, S = 10 nM Mg²⁺

E/H
C/H
G/H
A/H
H/E
H/C
H/G
H/A
H/H
H/H

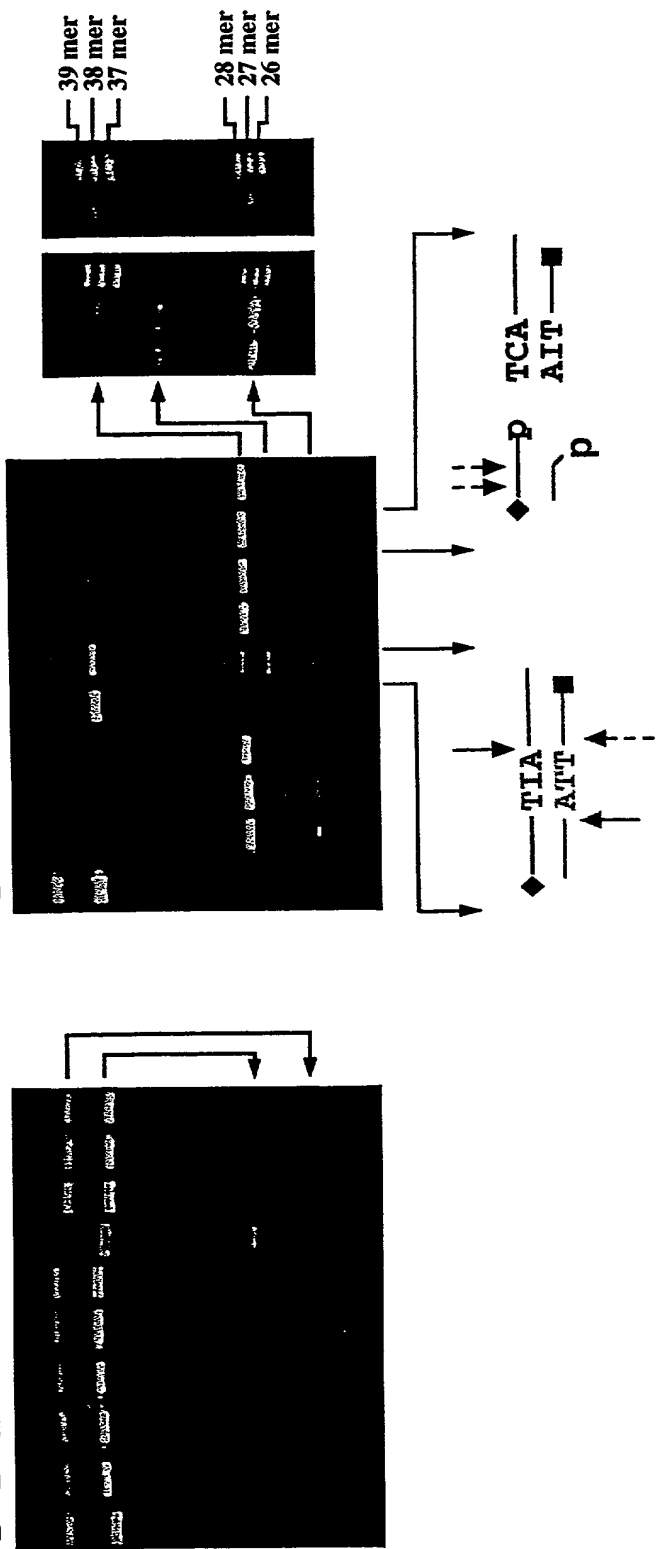
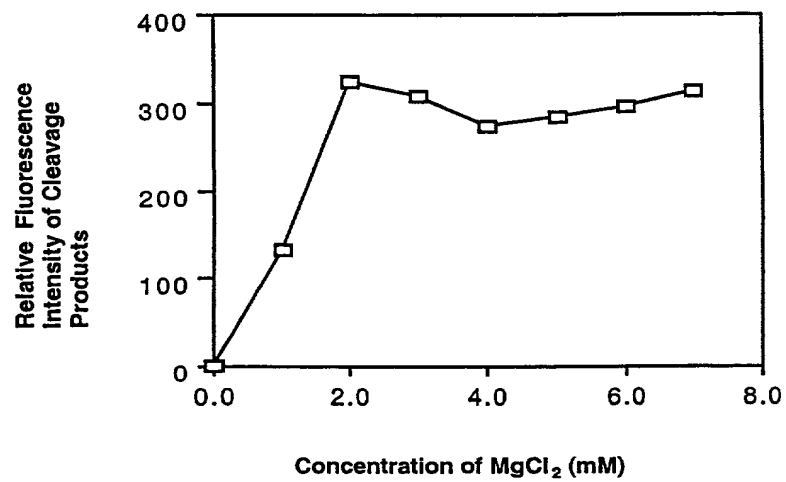


Figure 3

A



B

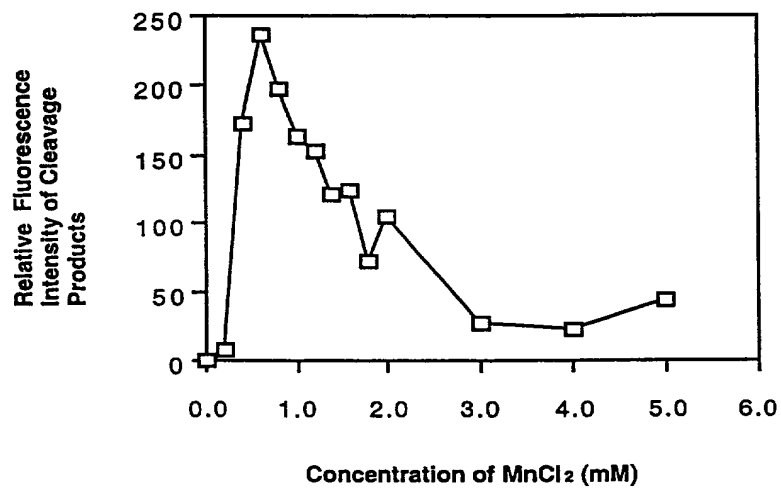


Figure 4

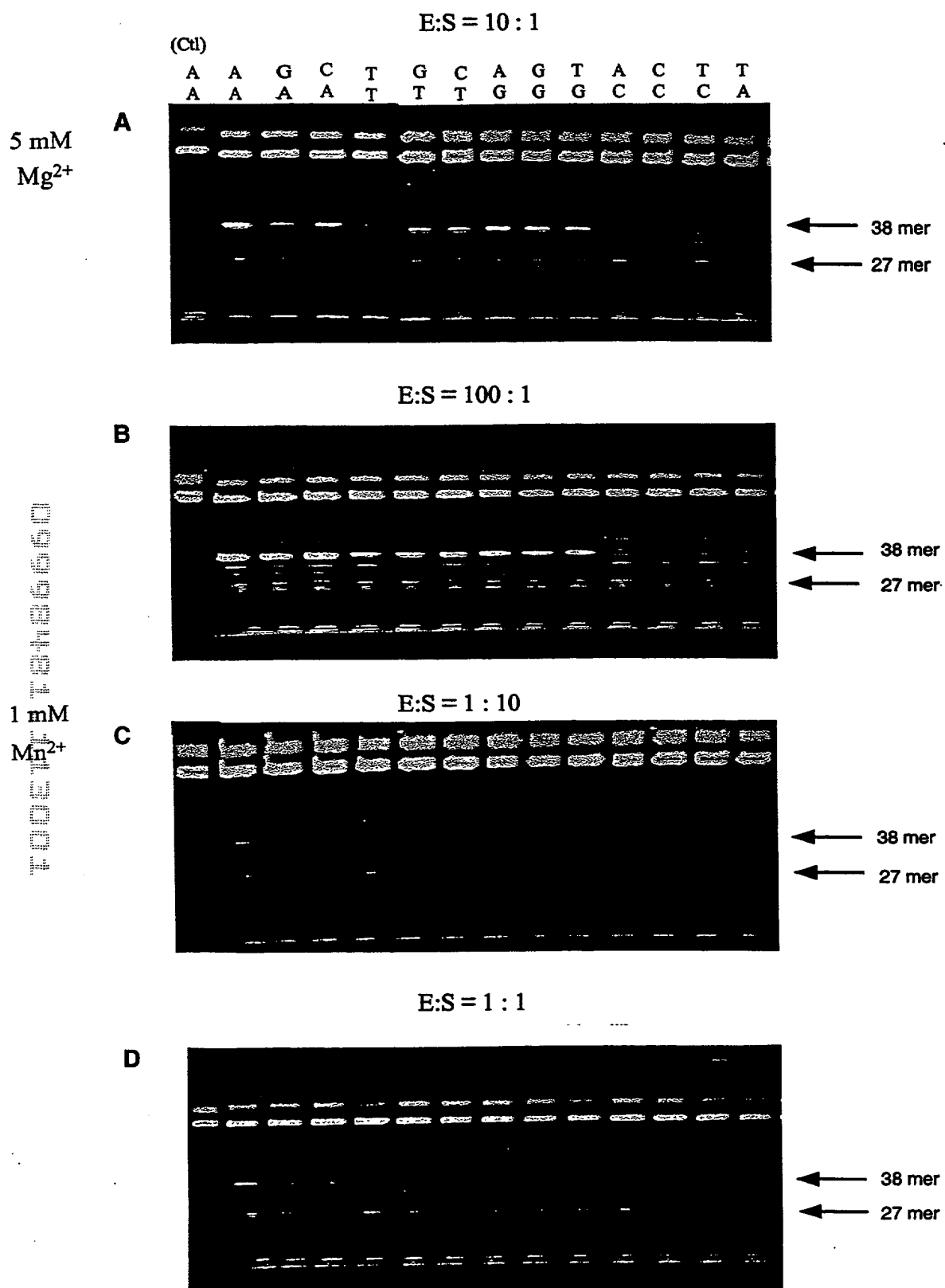


Figure 5

A

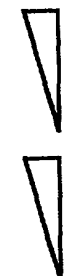
FOOETT" T848660

endo V -

endo V

$\frac{T}{AP}$

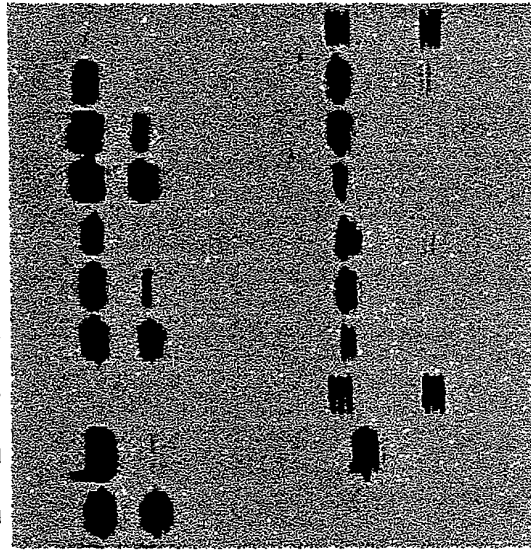
$\frac{T}{U}$



++ +

+++

1 2 3 4 5 6 7 8 9 10



B



$\frac{T}{AP}$

$\frac{T}{U}$

$\frac{I}{A}$

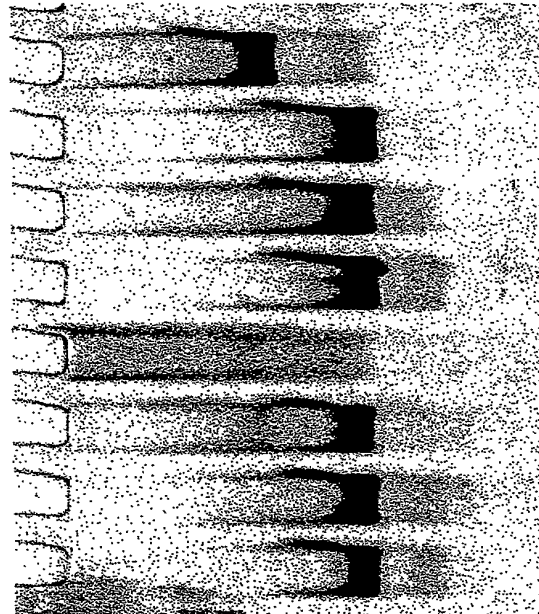


++ +

++ +

++ +

1 2 3 4 5 6 7 8



Bound

Free

Figure 6

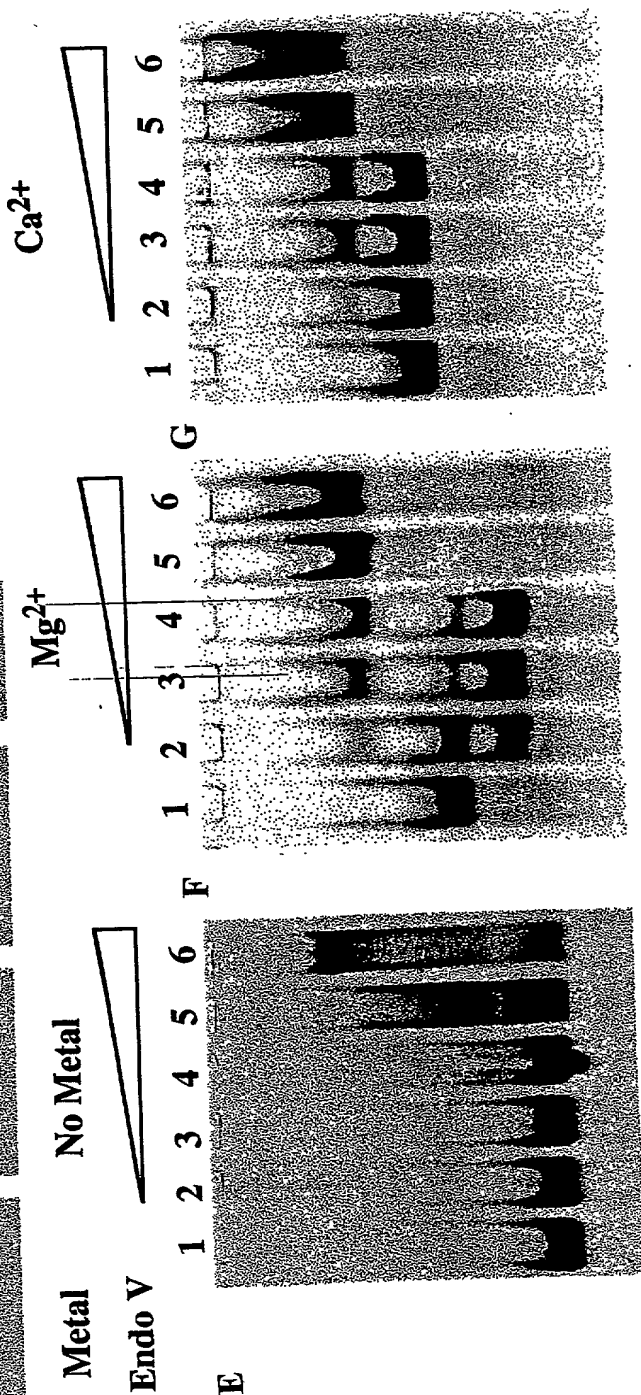
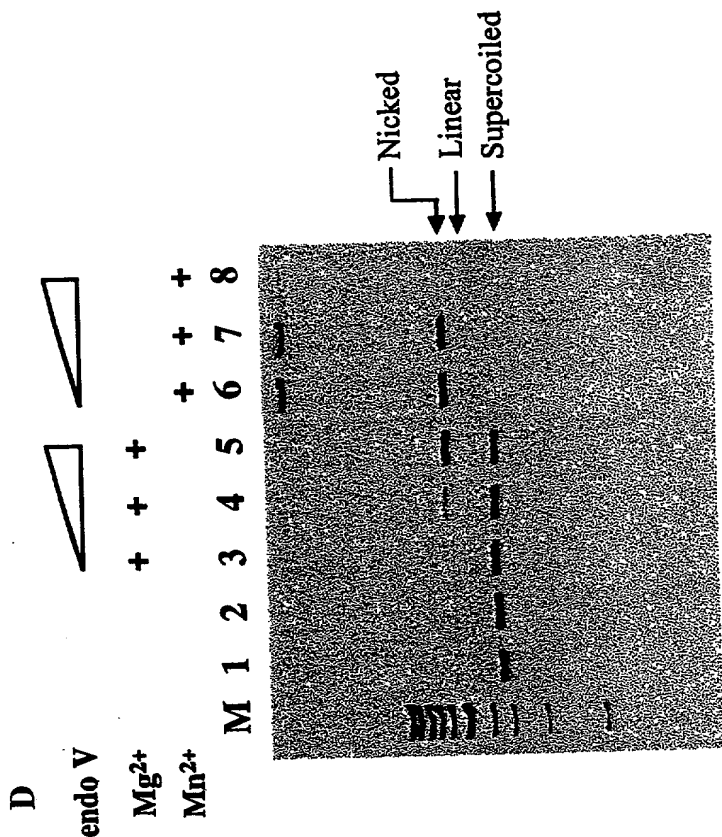
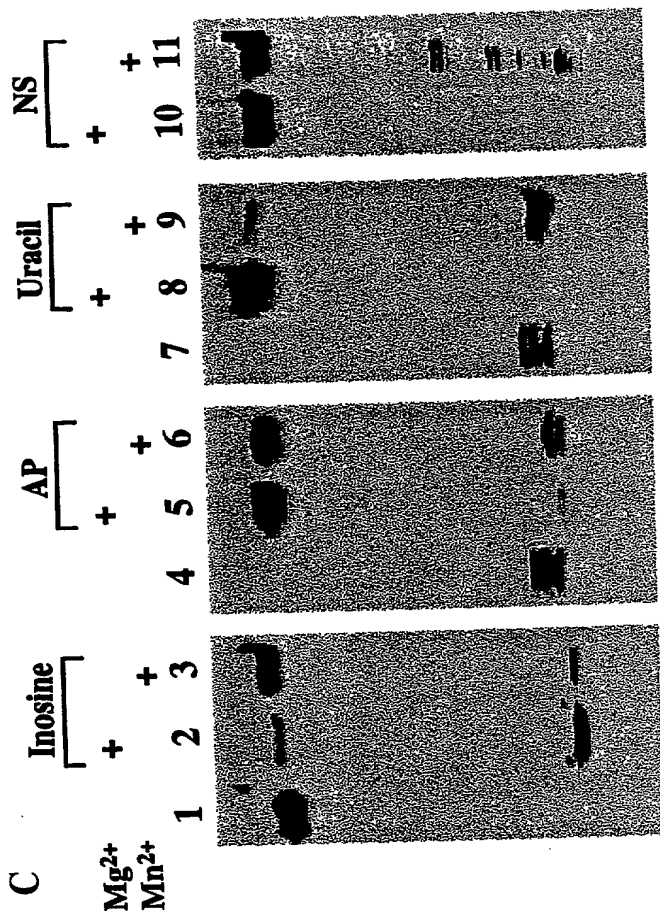


Figure 6 (cont.)

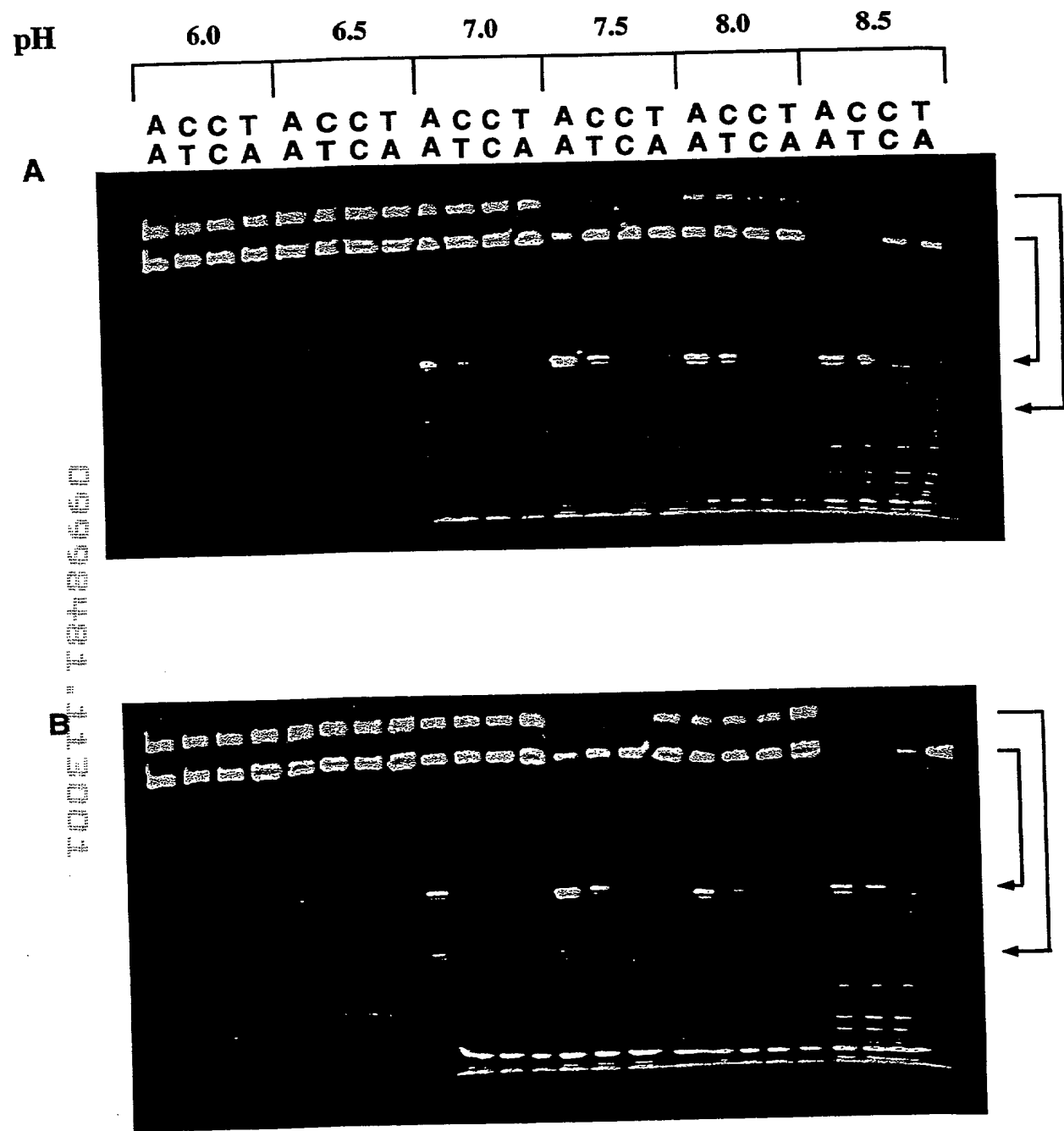


Figure 7.

Effect of salt

NaCl/mM

0

50

100

150

200

250

A	C	C	T	A	C	C	T	A	C	C	T	A	C	C	T	A	C	C	T	A	C	C	T
A	T	C	A	A	T	C	A	A	T	C	A	A	T	C	A	A	T	C	A	A	T	C	A

Mg²⁺

0992487

Mn²⁺

Figure 8

0990401 113001
T02 T T043660

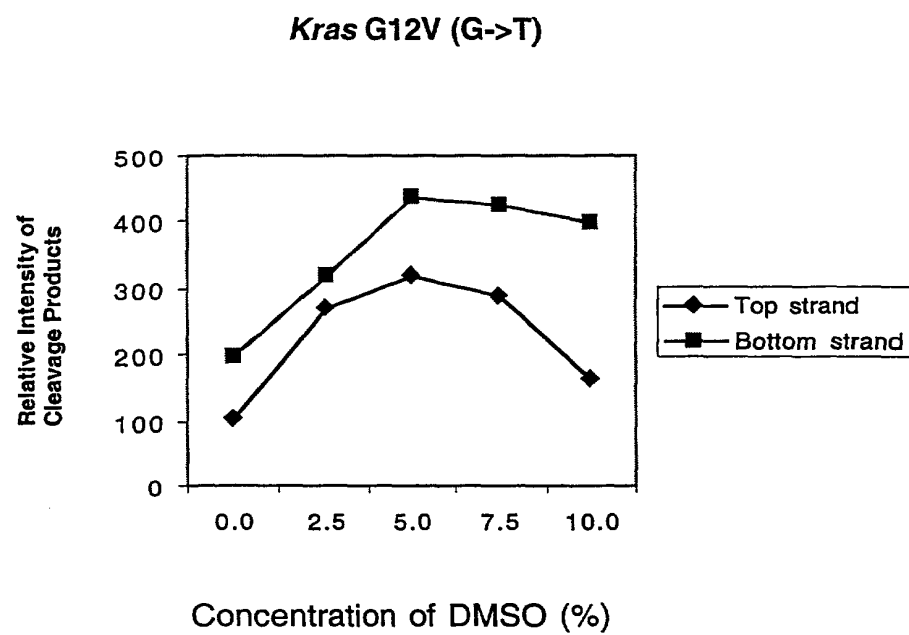
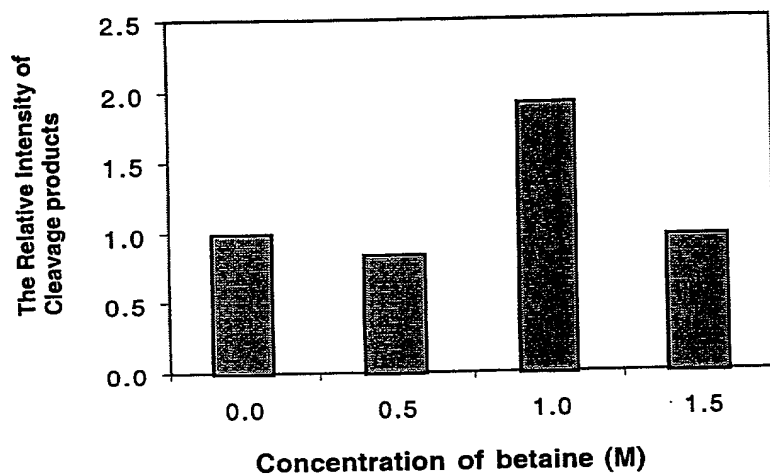


Figure 9

A

APC I1307K(T->A)



B

Kras G12V (G->T)

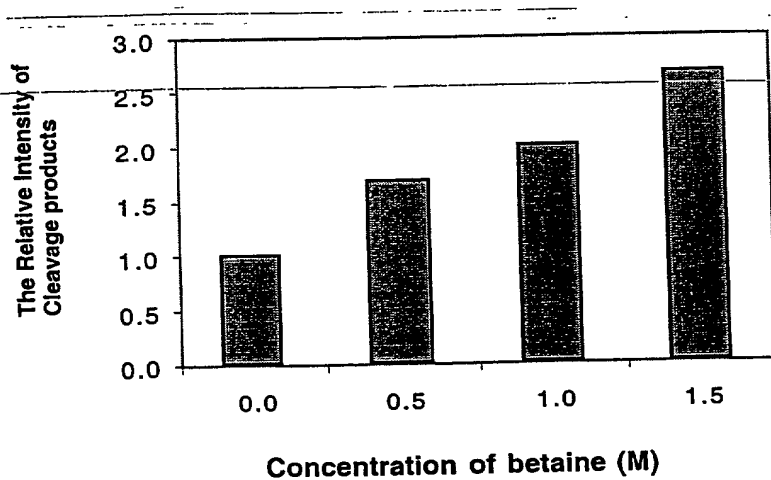
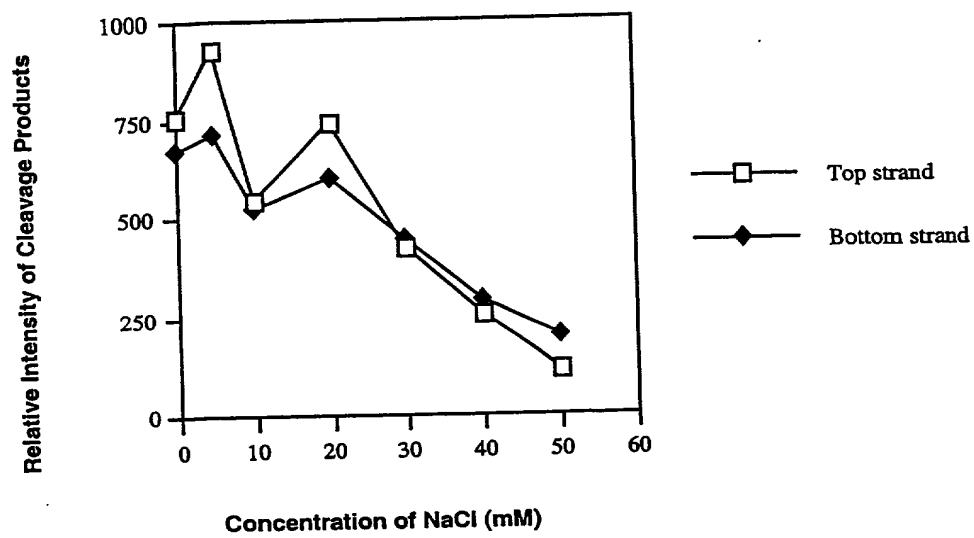


Figure 10

0990451 18786660

A

k-ras G12V (G->T)



B

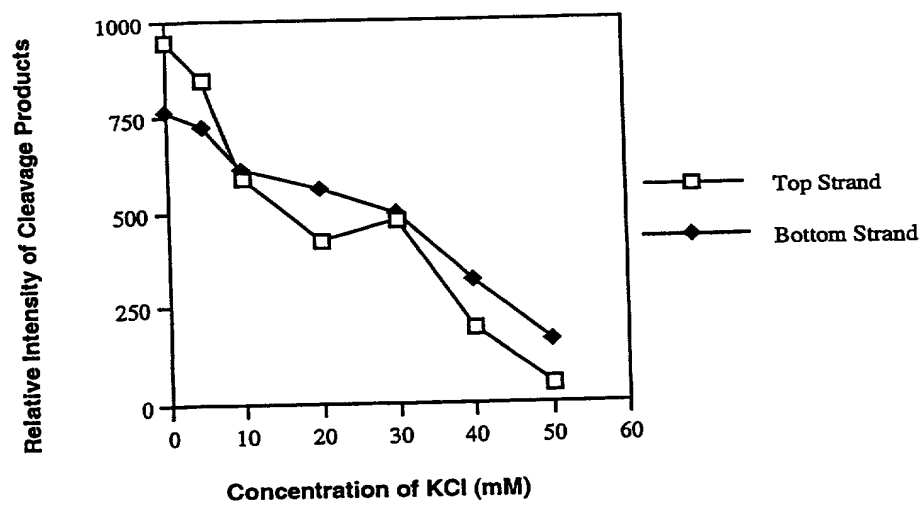


Figure 11

***k*-ras G12D (G->A)**

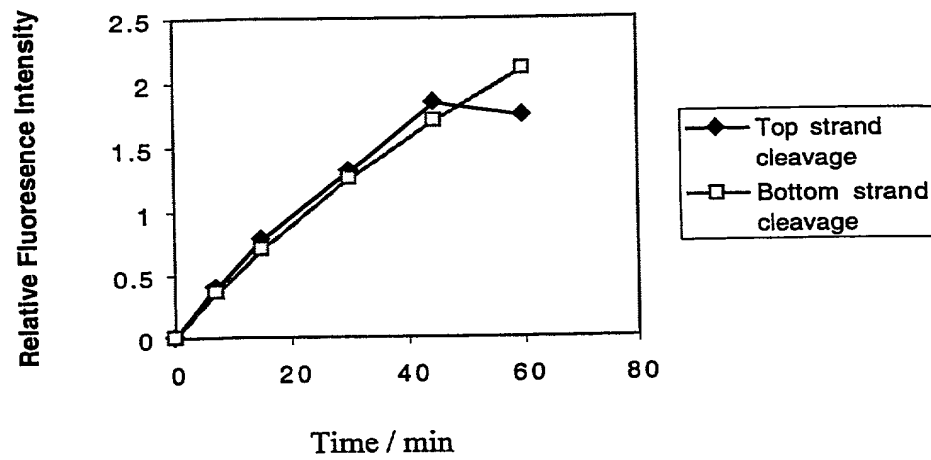
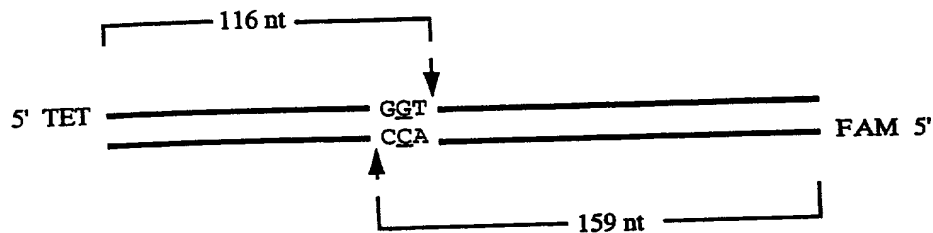


Figure 12

A



B

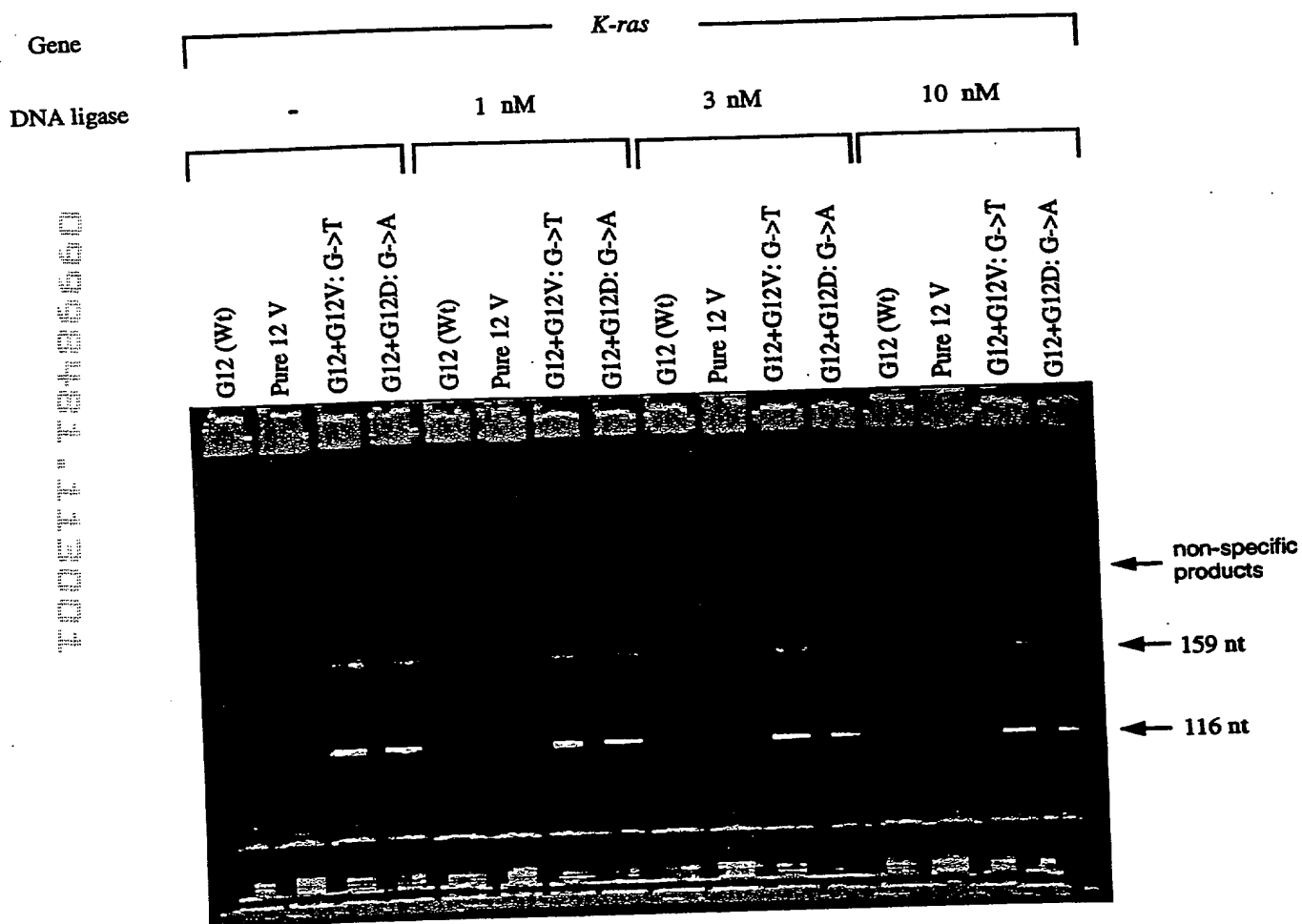


Figure 13

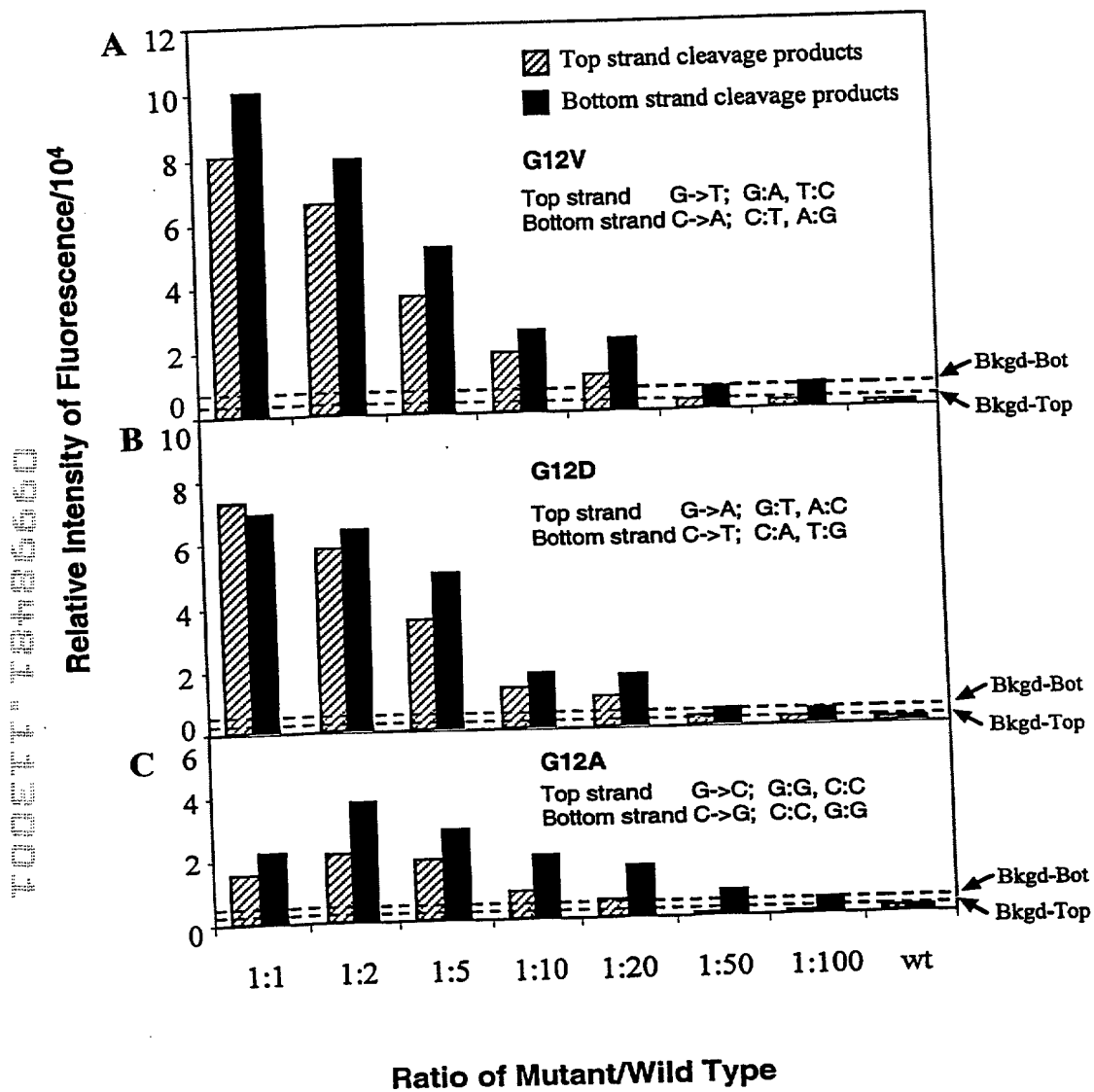


Figure 14

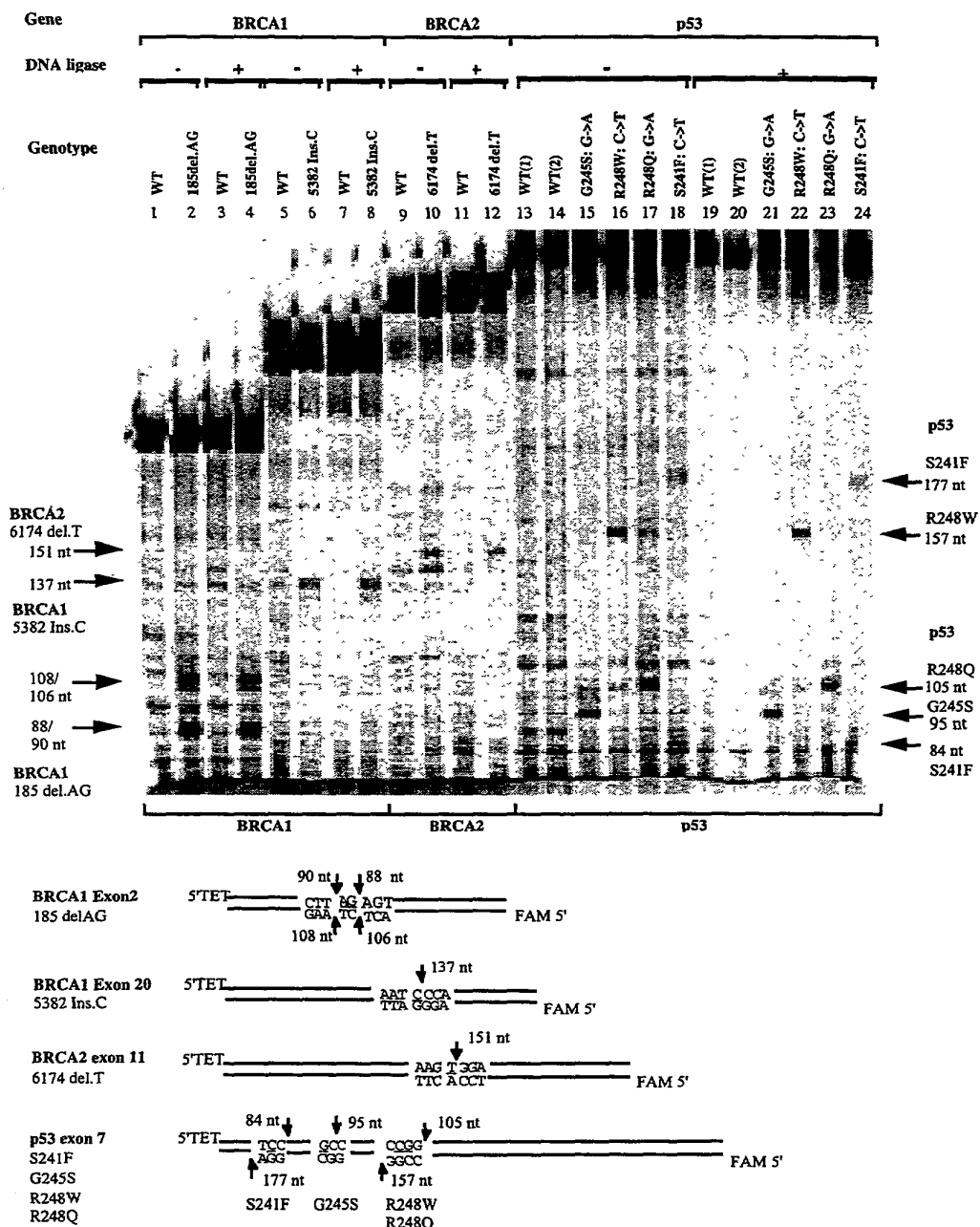


Figure 15

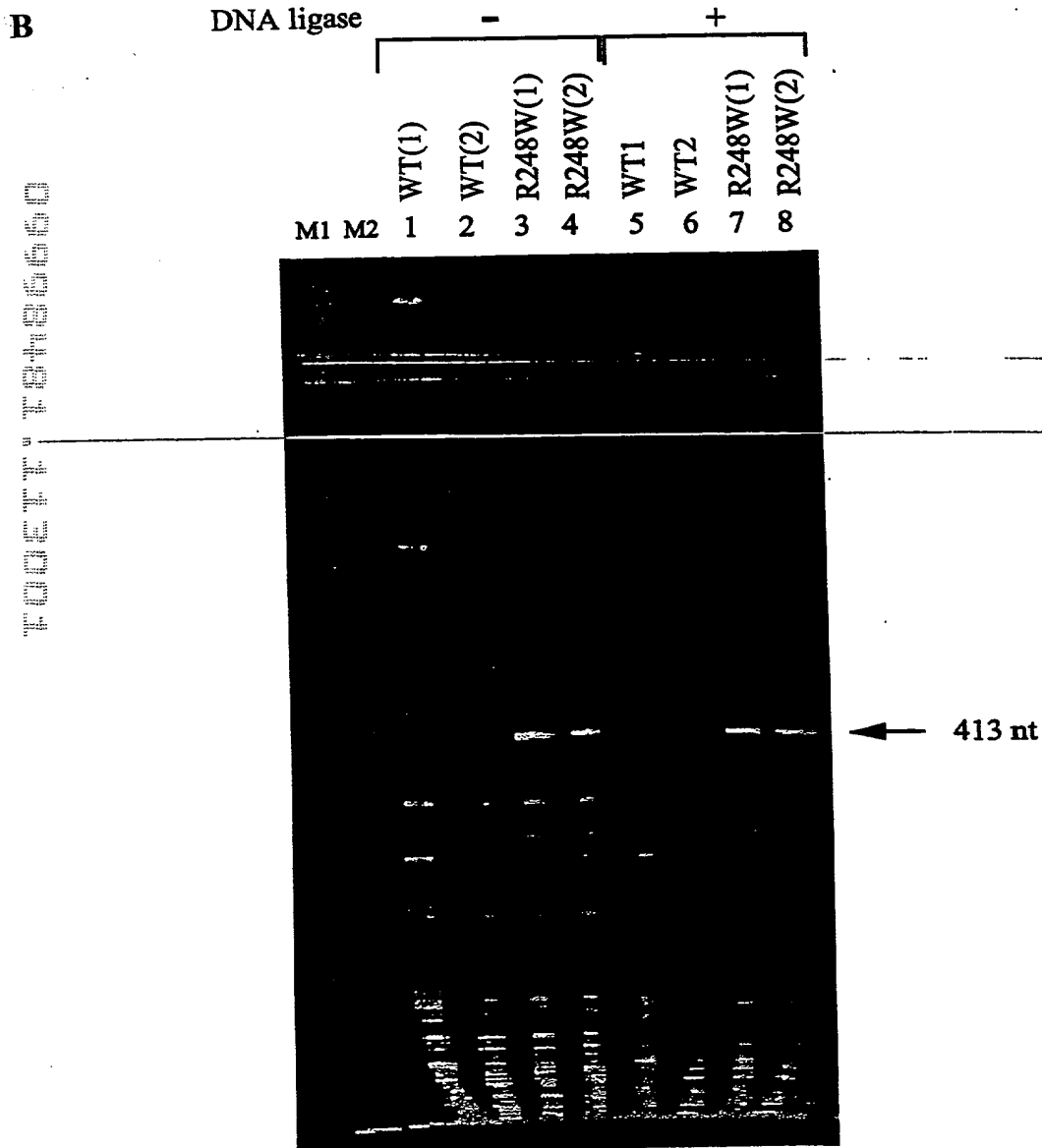
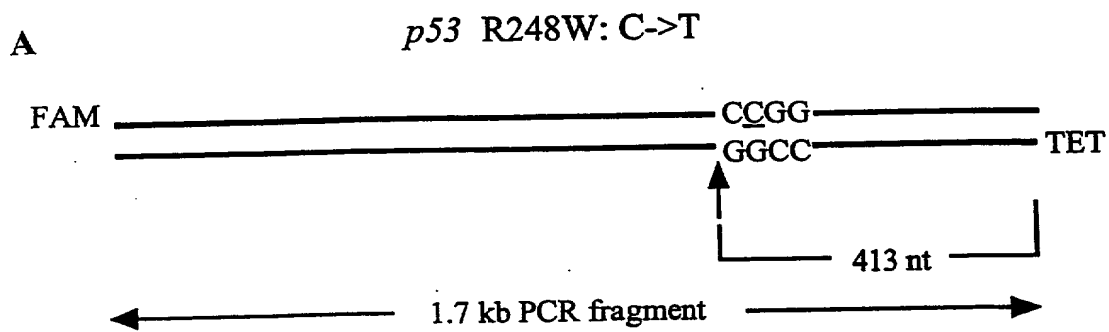
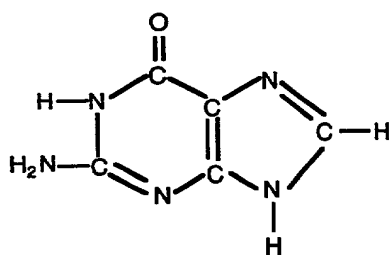


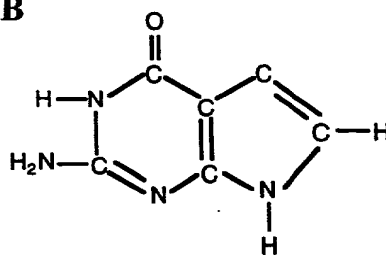
Figure 16

A



Guanine

B



7-deaza-Guanine

05938483.13004
T0067T" 18485550

C

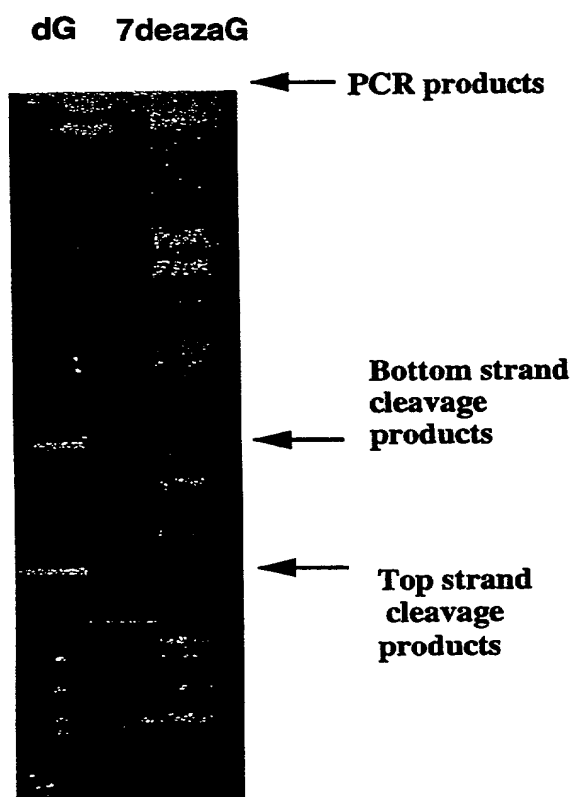
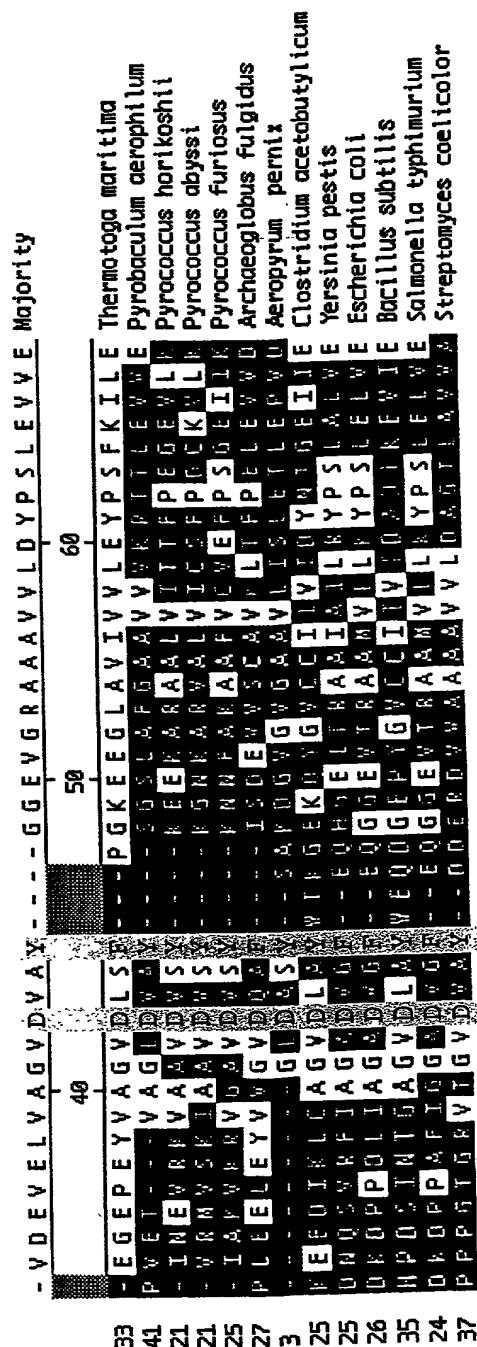
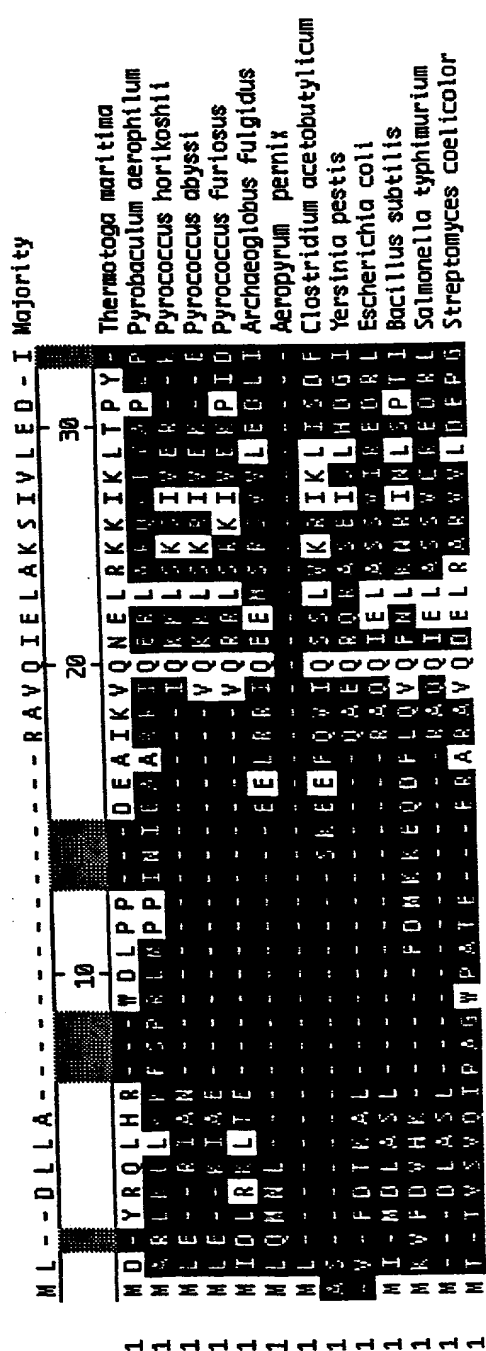
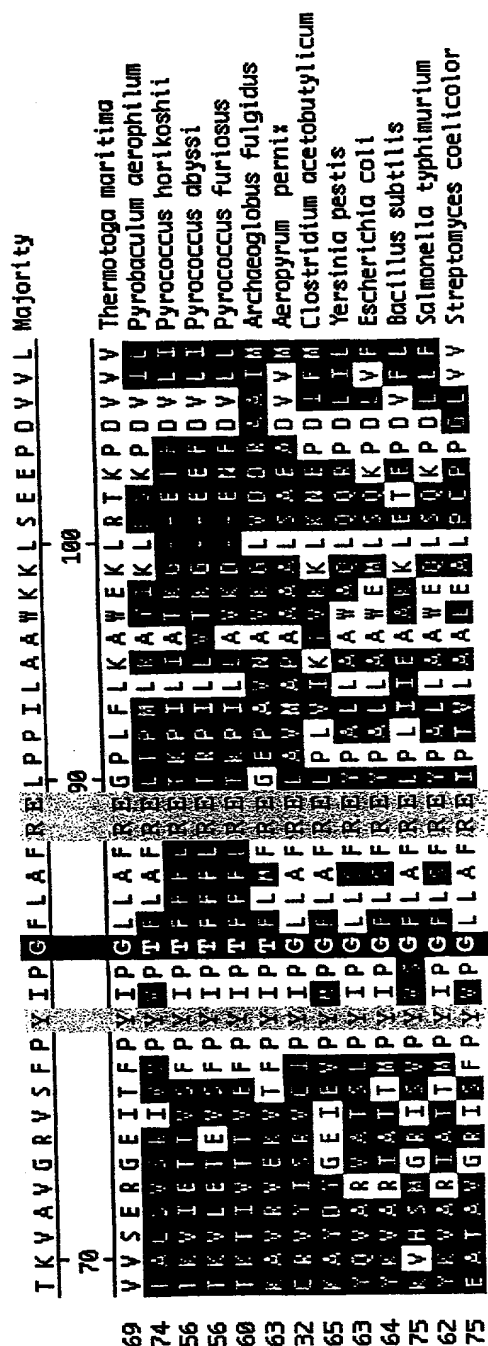


Figure 17

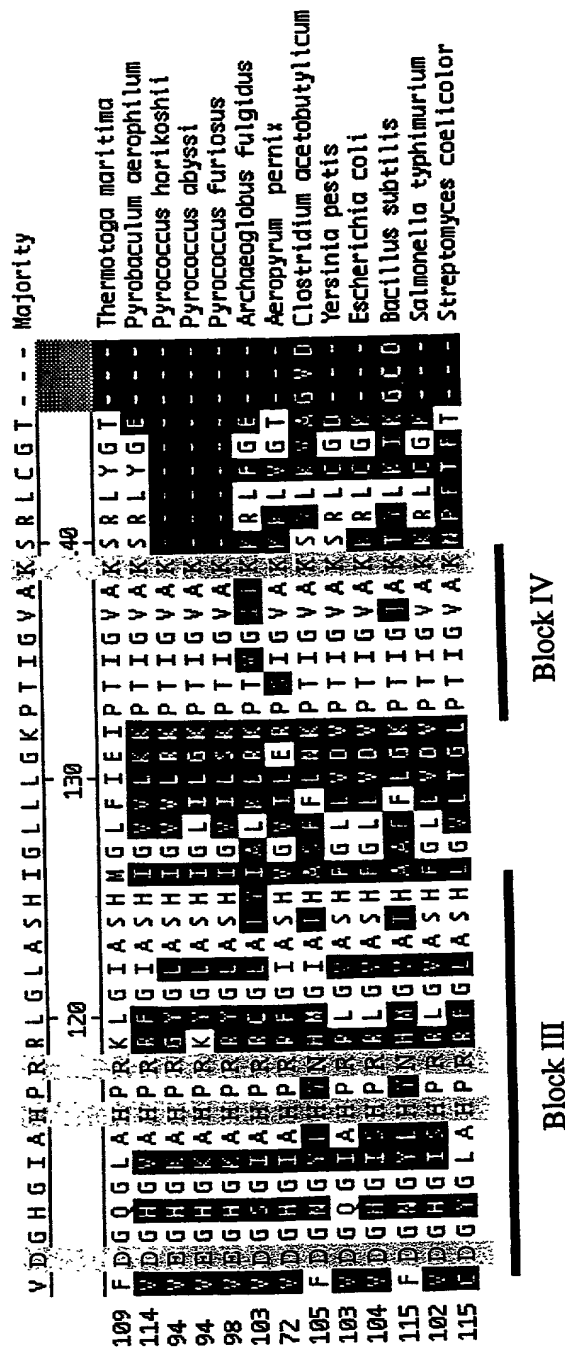


Block I

Figure 19



Block II



Block III

Block IV

Figure 19 (cont.)

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152	H	O	O	P	D	Y	P	R	G	S	I	S	P	L	L	A	G	A	E	E	V	G	G	R	A	V	R	T	R	G	W	F	P	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Block V

H R I T L D S A L A I V Q A L - L D - - G Y R L P E P T R L A D A L A K - R - - Majority										
190	200			210			220			
185	H L M D V E S S K R L I K A F T L P - - G R R I P E P T R L A H - I Y T Q R L K							Thermotoga maritima		
181	S Y A T L D E A A G L V A D L C F S G G V Y - - P P L P L A H E L A N - - -							Pyrobaculum aerophilum		
147	N L I T L E D A A T R I I A R A I - L D E S G G V Y - - P P L P L A D K L S R G R - - -							Pyrococcus horikoshii		
147	N M I T L E D A A R I I E E L - L D - G G V Y - - P P L P L A D K L S P G R - - -							Pyrococcus abyssii		
151	N L I T L E D A A R I I V E R L - L D E N G G V Y - - P P L P L A D K L S R G R - - -							Pyrococcus furiosus		
176	S Y I S P O S A L E L - L - T R E C L P - - G G W M P E P T R L A D A L A K I - - -							Archaeoglobus fulgidus		
142	H R I T L E F A A S I A R A T I E P - - G G W M P E P T R L A D A L A K I - - -							Aeropyrum pernix		
184	H R I T L E F A A S I A R A T I E P - - L I N N D S R L P E P T R L A D A L A K I - - -							Clostridium acetobutylicum		
179	H R V S V S S A L A V V Q R C - M A - - G G V R L P E P T R L A D A L A K I - - -							Yersinia pestis		
180	H R V S V S S A L A V V Q R C - M A - - G G V R L P E P T R L A D A L A K I - - -							Escherichia coli		
194	H R V S V S S A L A V V Q R C - M A - - G G V R L P E P T R L A D A L A K I - - -							Bacillus subtilis		
178	H R V S V S S A L A V V Q R C - M A - - G G V R L P E P T R L A D A L A K I - - -							Salmonella typhimurium		
191	H R V S V S S A L A V V Q R C - M A - - G G V R L P E P T R L A D A L A K I - - -							Streptomyces coelicolor		

Block VI

Figure 19 (cont.)

	Majority	
222	<i>Thermotoga maritima</i>	
213	<i>Pyrobaculum aerophilum</i>	
182	<i>Pyrococcus horikoshii</i>	
181	<i>Pyrococcus abyssi</i>	
182	<i>Pyrococcus furiosus</i>	
209	<i>Archaeoglobus fulgidus</i>	
180	<i>Aeropyrum pernix</i>	
214	<i>Clostridium acetobutylicum</i>	
216	<i>Yersinia pestis</i>	
217	<i>Escherichia coli</i>	
224	<i>Bacillus subtilis</i>	
215	<i>Salmonella typhimurium</i>	
226	<i>Streptomyces coelicolor</i>	

Figure 19 (cont.)

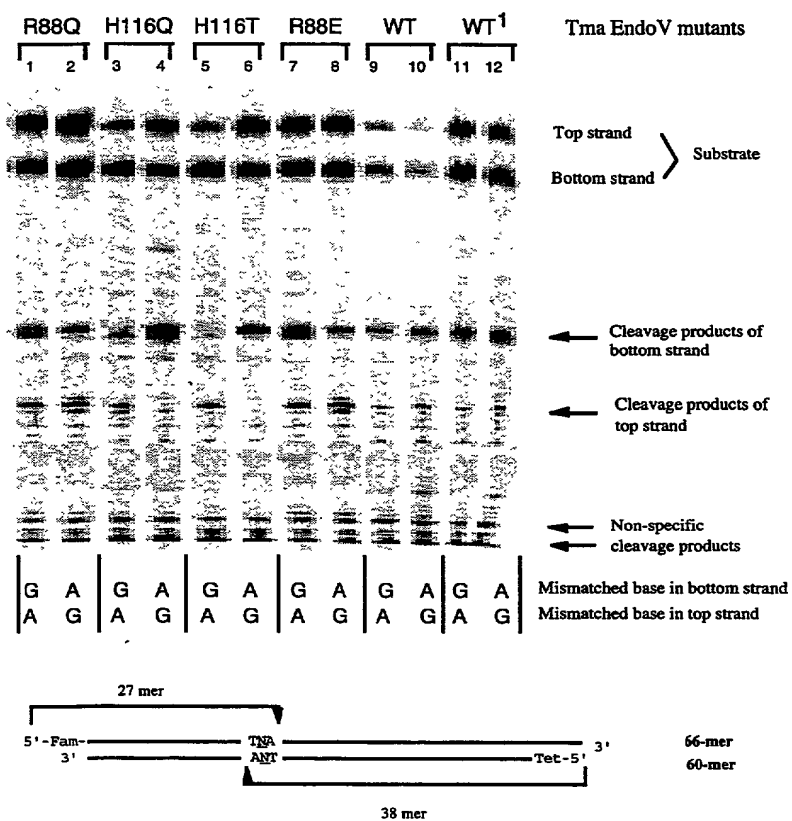


Figure 20

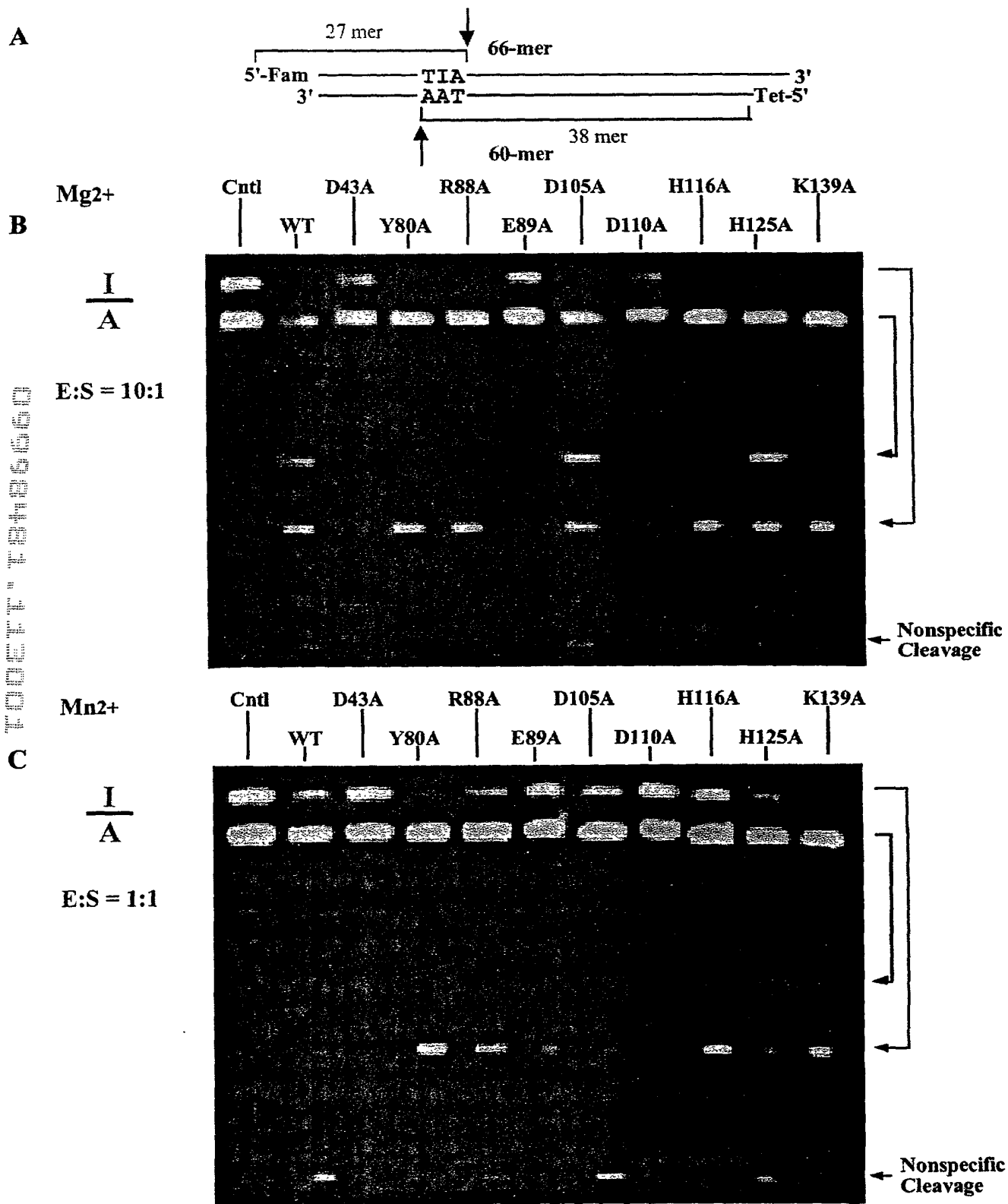


Figure 21

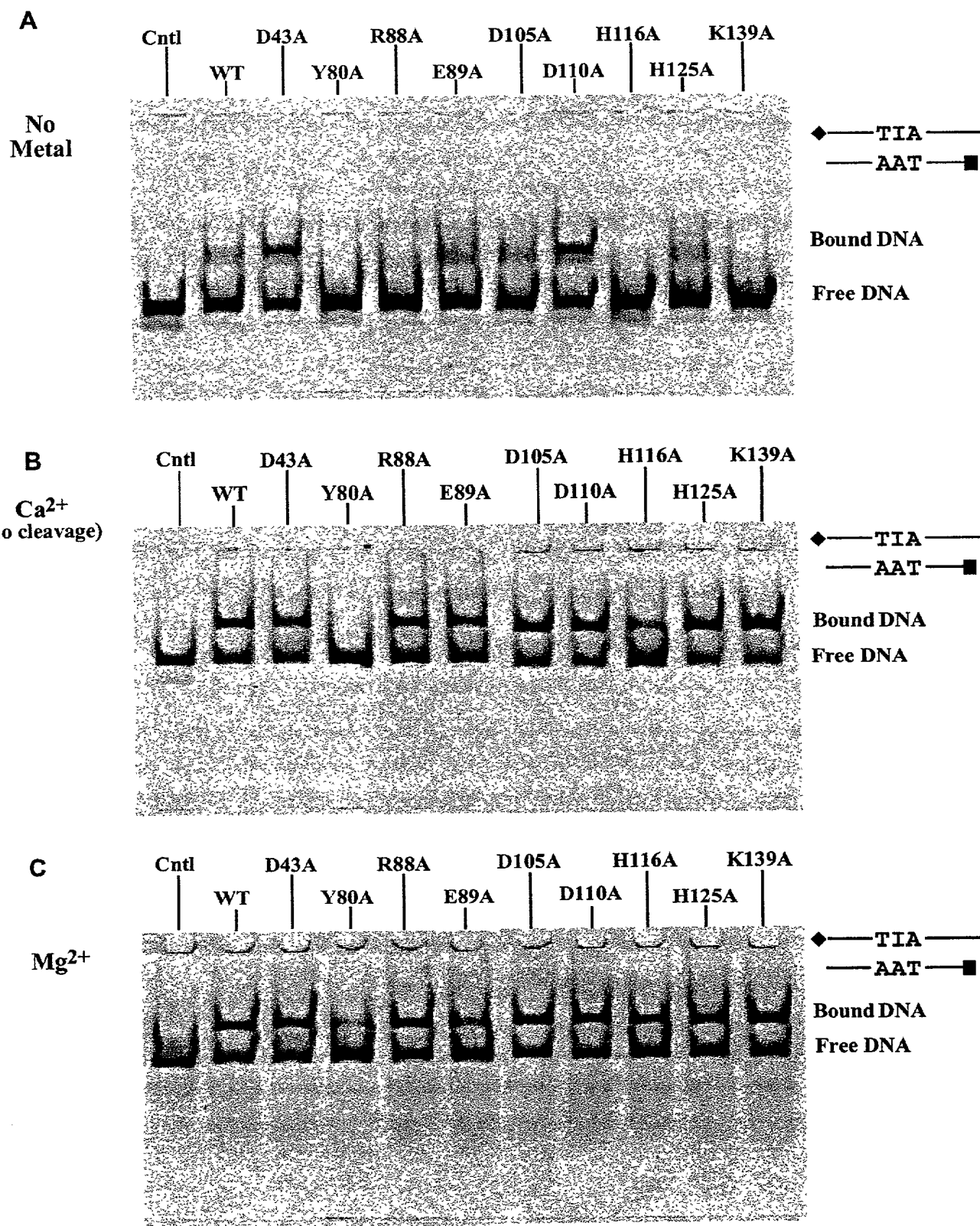


Figure 22

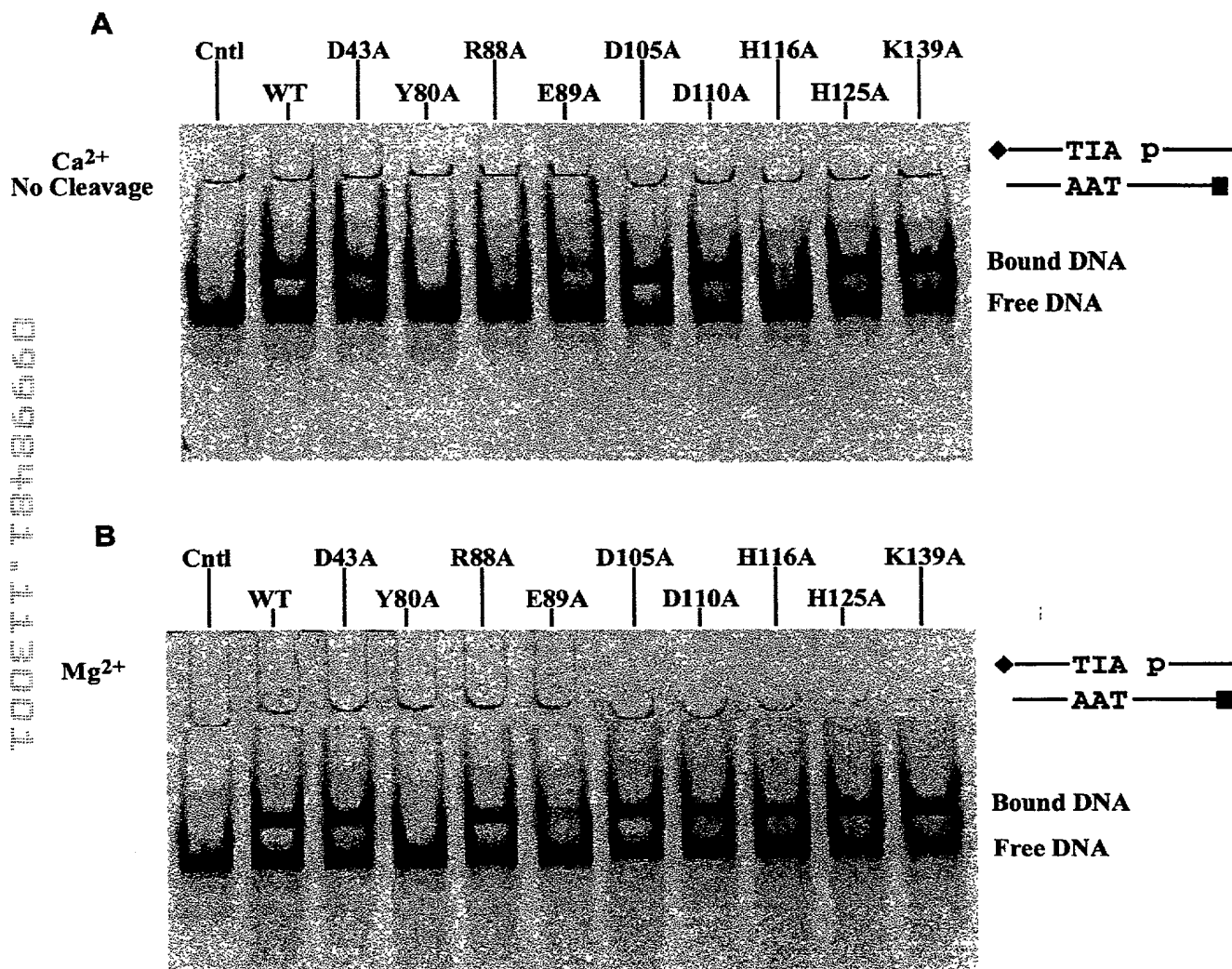
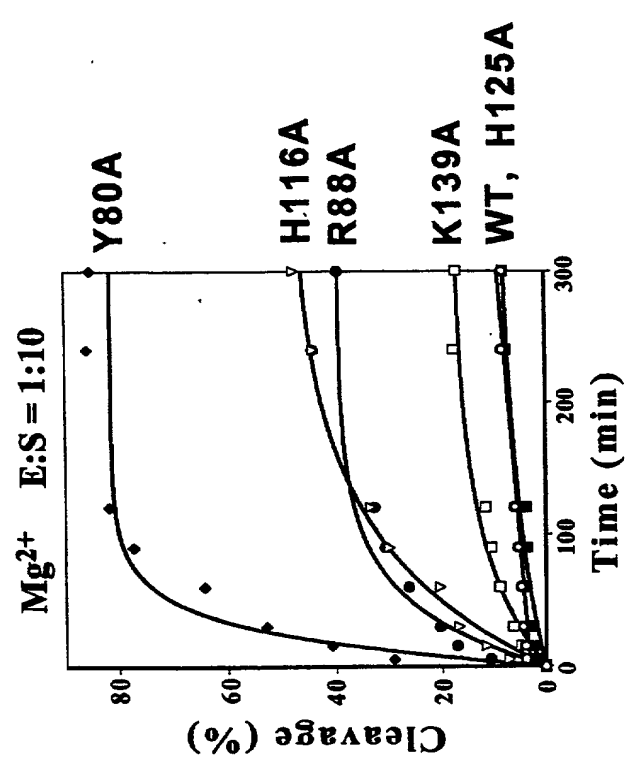


Figure 23

A



B

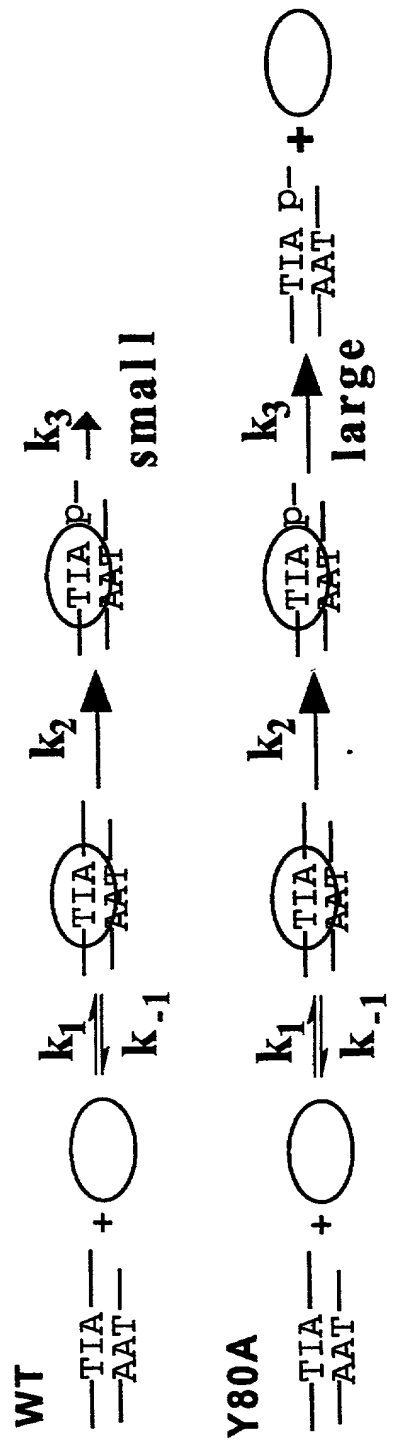
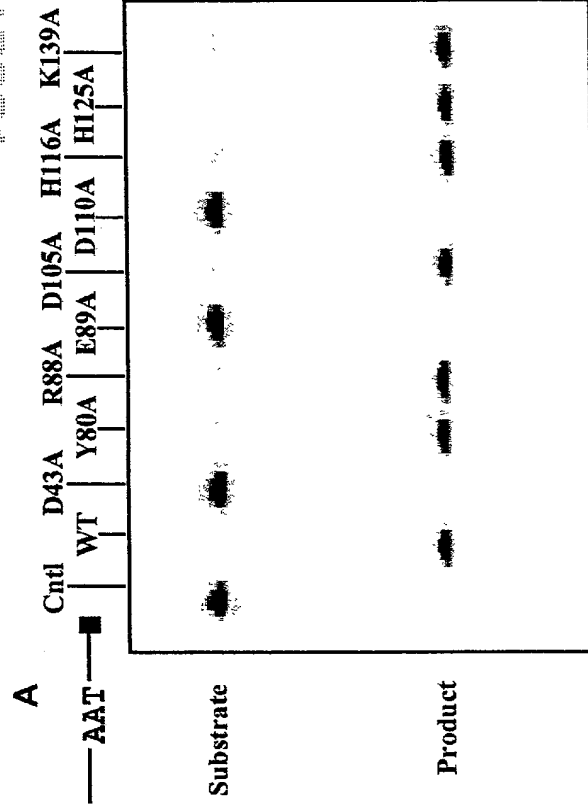
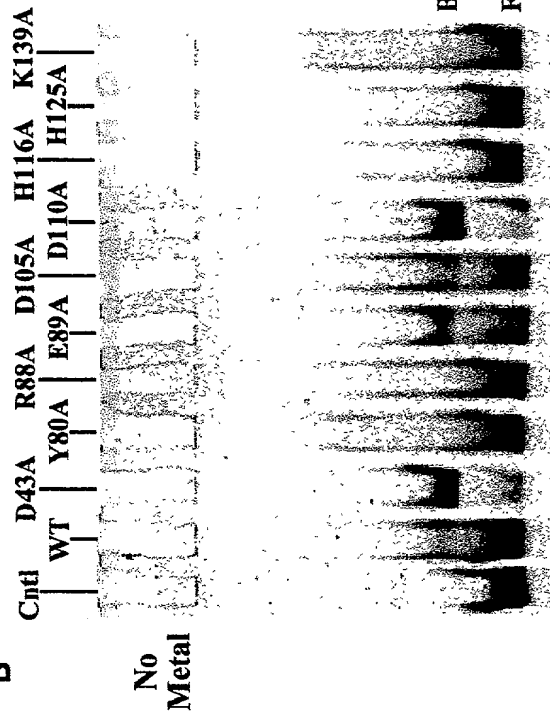


Figure 24

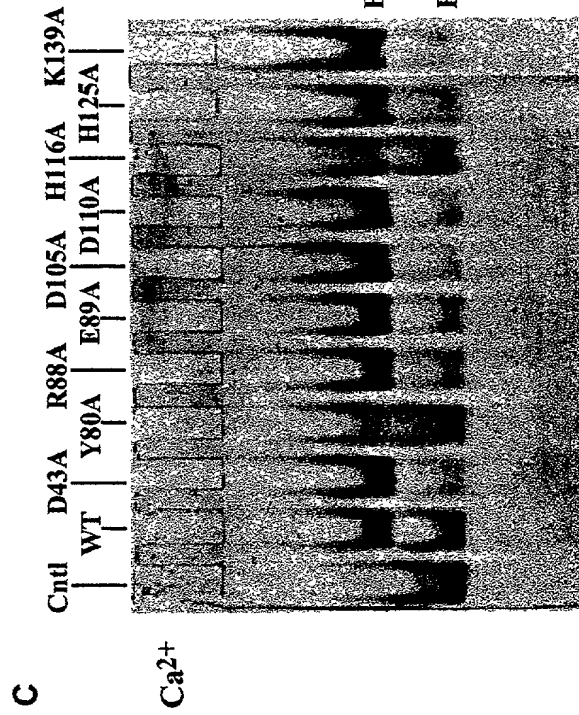
A



B



C



D

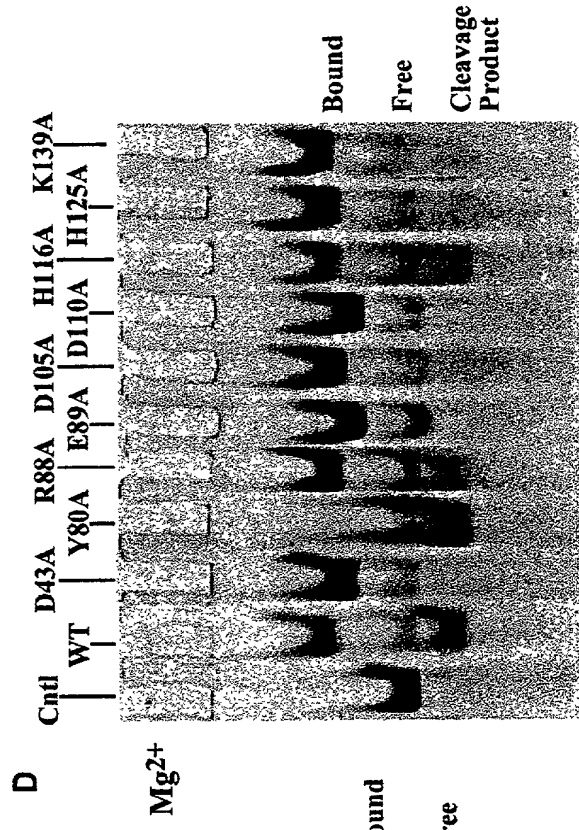
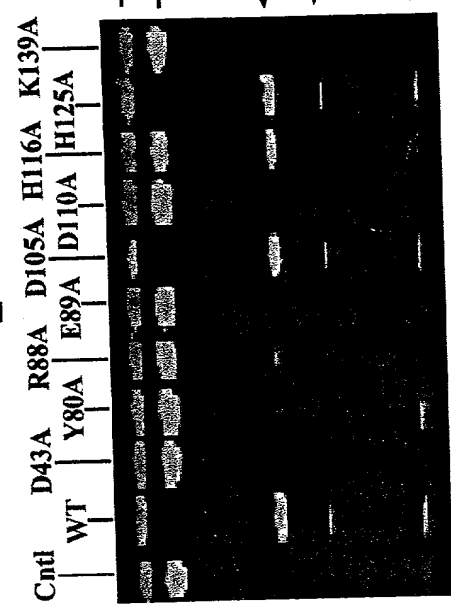


Figure 25

TGGCTTCTGTTGGGCTG

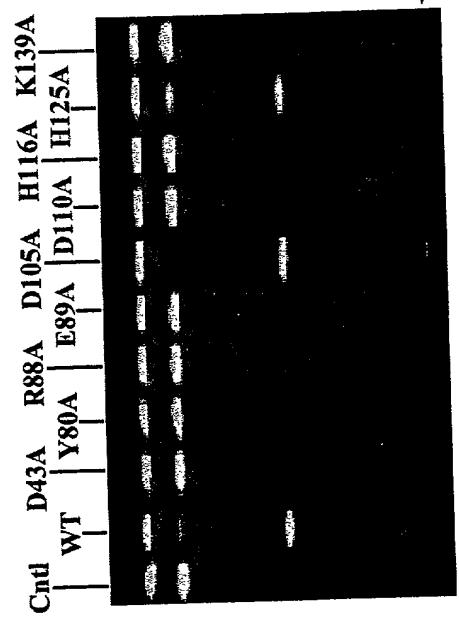
(G/U)
 ◆ —TGA—
 —AUT—■

A



(A/AP)
 ◆ —TAA—
 —A_T—■

D



(T/U)
 ◆ —TTA—
 —AUT—■

C

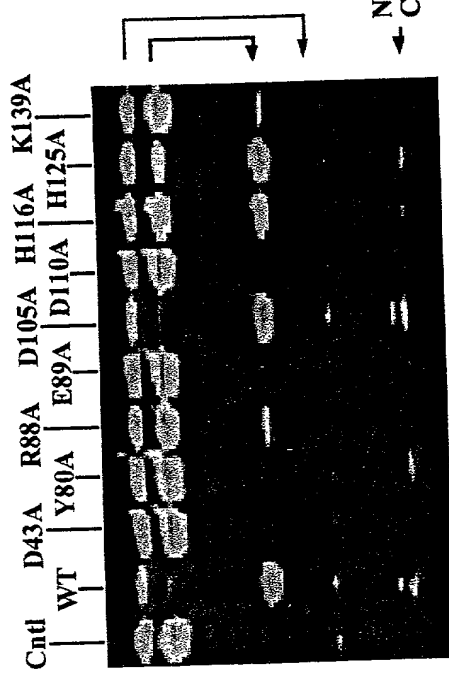


Figure 26